Fatty acid and lipopolysaccharide analyses of three *Heliobacterium* spp

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1. SUMMARY

The cellular fatty acids from *Heliobacterium chlorum*, *Heliobacterium gestii*, and *Heliobacillus mobilis* were analyzed. The fatty acid contents of the three organisms were essentially the same, consisting of large amounts of branched chain and some mono-unsaturated fatty acids. Neither a phenol-water nor a phenol-petroleum ether-chloroform extraction of whole cells yielded lipopolysaccharide.

3. MATERIALS AND METHODS

3.1. Organisms and culture conditions

*H. chlorum*, *H. gestii*, and *H. mobilis* were obtained from the laboratory of Prof. Howard Gest, Indiana University. Cells were grown in media described by Gest and Favinger [6]. *Stigmatella aurantiaca*, strain DW135, was grown as previously described [13]. *Escherichia coli*, strain W3101, was cultured in LB broth [14].

3.2. Chemicals

Fatty acid methyl ester standards were purchased from Applied Sciences Laboratories, Deerfield, IL.
3.3. Lipopolysaccharide extractions

Whole cells were extracted using the warm phenol-water procedure or the Galanos method [15–17].

3.4. Chemical assays

Total carbohydrate was measured using the phenol-sulfuric acid assay with glucose as the standard [18]. 2-Keto-3-deoxyoctonate (KDO) was measured using the method of Weissbach and Hurwitz as modified by Osborn [19,20].

3.5. GLC and GLC-mass spectroscopy (MS) of fatty acids

Methyl esters of fatty acids (FAME) from either whole cells or from the water phase of the phenol-water extracts were prepared according to Vinh et al. [21] except that boron trifluoride was substituted for boron trifluoride. Samples were analyzed with a Hewlett-Packard 5710A gas-liquid chromatograph using a 2 m × 2 mm stainless steel column packed with 10% diethylene glycol succinate on Chromosorb W AW-80/100 (Supelco). Isothermal analyses were performed at 160°C. FAME were identified by comparison of retention times to those of standards and by co-injection. Unsaturated fatty acids were also identified by their absence after bromination. Samples were also analyzed by capillary gas chromatography-mass spectroscopy using a Hewlett-Packard 5985 gas chromatograph-mass spectrometer containing a 30 m × 0.25 mm I.D. DB5 capillary column.

3.6. Gel electrophoresis

Samples were solubilized and, except for the E. coli samples, treated with proteinase K [21]. Electrophoresis was performed using 15% sodium dodecylsulfate polyacrylamide gels and lipopolysaccharide was detected using the silver staining method of Tsai and Frasch [22].

4. RESULTS AND DISCUSSION

4.1. Fatty acid composition

Forty to 60% of the whole cell fatty acids in the three organisms were branched chain, the predominant one being iso-palmitoleic acid (Table 1). Such large amounts of branched chain fatty acids are more typical of Gram-positive bacteria than they are of Gram-negative bacteria, although several Gram-negative bacteria have been reported to contain large amounts of branched chain fatty acids [12,23]. Two fatty acids, palmitoleic and iso-palmitoleic acid, comprised over 60% of the total fatty acids in all three organisms. The rest of the fatty acids were C14 and C18, some of which were mono-unsaturated.

Table 1

<table>
<thead>
<tr>
<th>Mol. wt.</th>
<th>Structure</th>
<th>Percent of total</th>
<th>H. chlorum</th>
<th>H. gestii</th>
<th>H. mobilis</th>
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<tr>
<td>242</td>
<td>Iso C: 13</td>
<td>0.636</td>
<td>1.049</td>
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<tr>
<td>254</td>
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<td>2.46</td>
<td>3.950</td>
<td>13.7</td>
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<tr>
<td>256</td>
<td>anteiso C: 14</td>
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<td>3.511</td>
<td>10.2</td>
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</tr>
<tr>
<td>268</td>
<td>C: 16:1</td>
<td>35.88</td>
<td>48.61</td>
<td>23.5</td>
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<tr>
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<td>2.846</td>
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<tr>
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<td>Iso C: 16:1</td>
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<td>27.39</td>
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<td>284</td>
<td>C: 17</td>
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<td>0.997</td>
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<tr>
<td>296</td>
<td>C: 18:1</td>
<td>12.12</td>
<td>9.689</td>
<td>&lt; 5.0</td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>C: 18</td>
<td>0.582</td>
<td>1.082</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Methyl ester, b Fatty acid. Identification of H. chlorum and H. gestii fatty acids was by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). Identification of H. mobilis fatty acids was by GC-MS. Fatty acids analyzed by GC were electronically quantitated. Not shown in the table are unidentified substances representing less than 1% of the total integrated area. ND = not detected.
Fig. 1. Silver-stained LPS patterns of whole cell extracts after SDS-polyacrylamide gel electrophoresis. Lanes: 1, 2, 11, *S. aurantiaca*; 3, 4, 12, *H. chlorum*; 5, 6, 13, *H. gestii*; 7, 8, 14, *H. mobilis*; 9, 10, 15, *E. coli*. 20 μg dry weight were loaded into lanes 1–10, and 40 μg into lanes 11–15.

4.2 *Lipopolysaccharide*

Most bacterial lipopolysaccharides are extracted into the aqueous phase of a phenol-water extract [15]. However, lipopolysaccharides that lack a major portion of the hydrophilic oligosaccharide chains are hydrophobic and can partition into both the phenol phase and the water phase. Thus the recovery of these hydrophobic lipopolysaccharides from the water phase is poor. We therefore also used the phenol-petroleum ether-chloroform extraction procedure of Galanos et al. which extracts certain lipophilic lipopolysac-
Charides into the phenol phase from which they can be precipitated by the addition of water [17]. Neither procedure yielded detectable lipopolysaccharide. The warm phenol-water method did extract polysaccharide as measured by phenolsulfuric acid assays and glucose determinations (Beck, H. 1987, M.A. thesis, Indiana University, Bloomington, IN). However, based upon the sensitivities of the assays, we conclude that there were less than 1.3 µg of KDO per mg (dry weight) of polysaccharide and less than 0.5 ng of fatty acids per mg of dry weight of polysaccharide. Furthermore, mild acid hydrolysis of the polysaccharide did not yield a precipitate which would be expected if there were lipid A attached via a KDO linkage to the polysaccharide. Nor was there any β-hydroxyfatty acid in whole cell fatty acids (Beck, H. 1987. M.A. thesis, Indiana University, Bloomington, IN). Fig. 1 shows the results of silver staining of a polyacrylamide gel which was loaded with 20 µg to 40 µg of the polysaccharide. The absence of significant silver staining supports the conclusion that lipopolysaccharide was not present. Thus, the polysaccharide extracted with the phenol-water mixture was clearly not lipopolysaccharide. These data strongly suggest that the cell wall of the Heliobacteriaceae is not of the typical Gram-negative type. This would be consistent with the placement of these organisms with the Gram-positive bacteria as suggested by the 16S RNA sequence data [7,11]. However, these data do not rule out the possibility of an unusual lipopolysaccharide that partitioned completely into the phenol phase and was not precipitable by the addition of water. Further study of these organisms is clearly warranted in order to more fully explore their cell wall chemistry.

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REFERENCES